Thermo Scientific Biological Safety Cabinets
Fumigation Methodologies

Technical Note

Due to its use in hazardous processes, the biological safety cabinet (BSC) is a highly regulated device. Generally speaking, regulatory bodies require the manufacturers of biological safety cabinets to provide a process for the user to biologically decontaminate the cabinet. This process is different from the surface decontamination methods used to prepare the BSC work area for the normal day-to-day use of the BSC. Decontamination or fumigation is used to decontaminate all areas of the biological safety cabinet, including interior plenums and components.

BSC fumigation is performed with a disinfectant in a gas or vapor state. The term "gas" refers to a chemical that is a stable gas at room temperature. A "vapor" refers to a chemical that is stable as a liquid at room temperature and converted either to a gas or microscopic droplets prior to its release into the cabinet. This chemical then penetrates all internal surfaces within the BSC, including through the HEPA filters. The chemical is typically one capable of killing bacterial endospores, which among bacteria, viruses, fungi, algae, and protozoa, are considered the most resistant to chemical disinfection.

There are four underlying needs for fumigating a BSC:

1. To ensure safety to service personnel who need to access potentially biologically contaminated areas of the BSC, e.g., for HEPA filter changes or motor replacement.
2. To help prevent contamination of samples being processed inside a BSC, e.g., changeover of pharmaceutical lot production run.
3. To prevent outbreak of highly infectious agents that could harm laboratory personnel, before non-routine service or as a part of a regular contamination control regimen, e.g., BSL-3 or -4 facilities.
4. Fumigation should also be performed prior to relocation of the biological safety cabinet in order to protect the movers and reduce the potential release of biohazardous material during the transportation process.

NOTE: The fumigation methods required by the regulatory bodies and described herein are directed to the biological hazards contained and controlled by the biological safety cabinets. Cabinets used for work with chemical or radiological hazards may require additional procedures to address the risks from those materials. With all fumigation procedures, local authorities should be consulted prior to performing any procedure. Depending on the region, there could be legal issues to address regarding training of personnel and use of appropriate personal protective equipment (PPE).

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Frequency of Fumigation

The frequency of fumigation depends on requirements of the institution and the need for fumigating.

In most general research facilities, standard surface cleaning procedures can prevent contamination. Typically, the only time fumigation is required is at a major service interval, such as when HEPA filters are consumed and require replacement. Often a filter change is only required once during the cabinet's life cycle. Fumigation will also be required when a non-functioning component located in a potentially contaminated plenum area requires replacement.

In some industry segments there is a need for fumigating the BSC in between batches, lots or experiments. Lots or batches could change as often as once per week or once per month, requiring 10-30 decontaminations during a given year. Because of this extreme frequency of fumigation, it is important to pay close attention to material compatibility issues. The more frequently the BSC is exposed to corrosive gases and vapors, the more likely for materials inside the BSC to break down. Component manufacturers generally do not warrant these items against repeated exposure to disinfectants.

Fumigation Procedures

The three most commonly used chemicals in BSC fumigation are formaldehyde (CH_2O), chlorine dioxide (ClO_2), and hydrogen peroxide (H_2O_2).

Each chemical has advantages and disadvantages in this application, and is described in more detail in the following sections.

Formaldehyde *Chlorine Dioxide* *Hydrogen Peroxide*

Formaldehyde

Formaldehyde is an organic compound with the chemical formula CH_2O. In its steady-state it is a colorless gas with a pungent odor. Commercial solutions of formaldehyde in water are called formalin. Formaldehyde has been shown to produce a 6-log reduction of the spore species Bacillus atrophaeus, typically used as a biological indicator, for certain applications. Fumigation with formaldehyde gas is performed either by vaporizing formalin solution or by depolymerization of solid paraformaldehyde.

Health and Safety

While the permissible exposure level (PEL) for formaldehyde is 0.75 ppm, the classification of this agent as a carcinogen makes it extremely important to take care to avoid any level of exposure.* Some regions have additional requirements, for example the US Occupational Safety and Health Administration (OSHA) describes access restrictions, training requirements, and other actions for areas exposed to formaldehyde in excess of 0.1 ppm. Therefore, it is usually recommended that all non-authorized personnel leave the room during the formaldehyde fumigation process.

Effectiveness

The use of formaldehyde for BSC fumigation, as described in NSF/ANSI 49, has a long tradition in North America. In 2000, the European Standard for biological safety cabinets (EN 12469:2000) was published and included Annex J (informative) Recommendations for decontamination, cleaning and fumigation of BSCs and filters, which also describes a method for fumigation using formaldehyde. This procedure has many similarities to the NSF/ANSI 49 procedure.

Material Compatibility

As the industry has broadly used formaldehyde decontamination for decades, the general material compatibility characteristics of formaldehyde fumigation of BSCs are good.

Ease of Use/Cycle Time

One of the major deficiencies of formaldehyde fumigation is the long cycle time required to complete the process. Paraformaldehyde is heated in a pan over a burner inside the BSC chamber to create formaldehyde gas, which is circulated through the BSC for 6-12 hours. After the formaldehyde contact time, the formaldehyde gas must be neutralized. This is usually done using ammonia created by heating ammonium bicarbonate or ammonium carbonate. This process of decontamination with formaldehyde and neutralization with ammonia may leave a residue on the surface that must be hand-cleaned. The entire process takes 18-24 hours to complete, possibly limiting access to the room while the cycle is in process.

EN 12469 recommends vaporizing 60ml of 36% formalin and 60ml of water per cubic meter of cabinet volume. NSF/ANSI 49 recommends depolymerizing 0.30 g/ft3 (11 g/m3) of space to determine the gram weight of paraformaldehyde required.

An improved formaldehyde fumigation process for treatment of HEPA filter in biological safety cabinets is available on request. This process is approved by a German governmental institution.

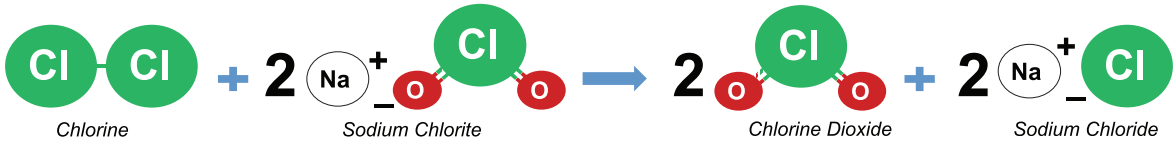
* Adapted from Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (IFA) that contains a database of occupational limit values for hazardous substances gathered from various EU member states, Australia, Canada, Japan, New Zealand, Singapore, South Korea, Switzerland, and the United States. For more information visit: http://www.dguv.de/ifa/en/gestis/limit_values/index.jsp

** Supplemented to the listing of verified and accepted disinfectants and disinfection procedure by the Robert Koch Institute, Germany (15th Edition, 31.01.2012)

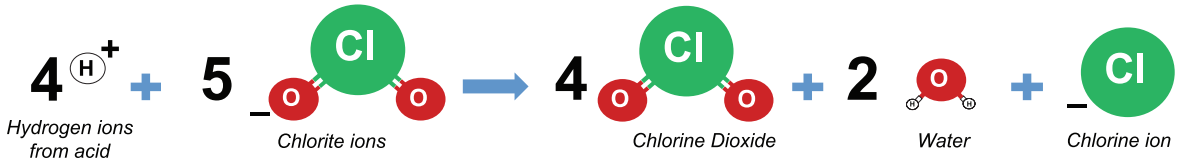
Chlorine Dioxide

Chlorine dioxide (ClO_2) is a synthetic, green-yellowish gas with a chlorine-like, irritating odor. Chlorine dioxide is a small, volatile and very strong molecule. Chlorine dioxide is an unstable gas that dissociates into chlorine gas (Cl_2), oxygen gas (O_2) and heat. This gas rapidly expands and penetrates the crevices of the area to be decontaminated.

Two typical reactions have been used for BSC fumigations. The first involves the reaction of chlorine gas with the salt sodium chlorite:



The second reaction, requiring the presence of water, involves the reaction of chlorite ions from sodium chlorite reacting with acid:



Health and Safety

The PEL for chlorine dioxide has a high toxic potential at only 0.1 ppm.* It is very important that the BSC framework is tightly sealed during this process. It may be necessary to limit access to the laboratory during the process to minimize exposure to workers. Chlorine dioxide is easily detected in the air by its identifiable smell.

Effectiveness

Chlorine dioxide and other chlorine-based chemicals are known to be extremely effective in decontaminating microorganisms and spores. Because it is a gas, it can easily expand to all areas of a BSC. Its gaseous properties also allow the penetration of plastics. A validated procedure for fumigation using chlorine dioxide is provided in NSF/ANSI 49, Annex G.

Material Compatibility

Chlorine dioxide is highly corrosive and is known to react negatively with steel and plastics. Over time and after multiple fumigations, this process is likely to cause deterioration of key BSC components which will be impacted from repeated or prolonged exposure to the gas.

Ease of Use

The chlorine dioxide fumigation procedure is faster than formaldehyde. If a BSC is ducted to an external exhaust system, the entire process could take less than three hours to complete. Non-exhausted BSCs will take slightly longer, as the chlorine dioxide must be removed from the cabinet by an activated carbon scrubber before the BSC can be deployed back into operation.

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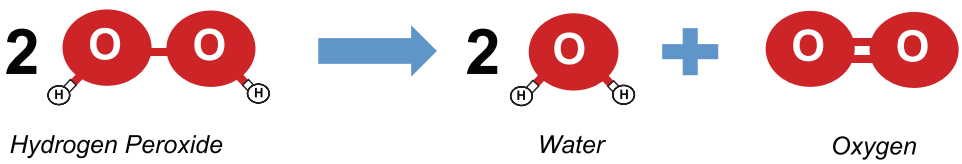
Hydrogen Peroxide

Hydrogen peroxide is a clear, colorless liquid, and more viscous than water. It is most commonly available as a solution in water. For consumers, it is usually available from pharmacies at 3 and 6 wt% concentrations. For laboratory use, 30 wt% solutions are most common.

For fumigation of biological safety cabinets, this method has to be validated for the model and size, according to the Annex G of NSF ANSI 49. In EN 12469 it is mentioned as an alternative to the formaldehyde fumigation process.



This method is fast, in contrast to a number of other fumigation techniques, and does not produce environmentally harmful end products. The hydrogen peroxide generators typically used in this process also provide a recordable printout of process parameters which aids in validation of the procedure. As illustrated above, only water and oxygen are retained as residual products after a fumigation cycle.

Fumigation with hydrogen peroxide first requires a procedure to dehumidify the BSC to prevent condensation. Once the humidity and temperature levels are stabilized, a 35% hydrogen peroxide solution is vaporized via a generator and released into the BSC. Vaporized hydrogen peroxide is very sporicidal at low concentrations (typically 0.1-3 mg/L at 25°C). During the inactivation phase, the hydrogen peroxide concentration is maintained at a maximum concentration level. The percentage of hydrogen peroxide that is "consumed" by absorption or decomposition is resupplied to the system. Finally, the hydrogen peroxide vapor is removed from the system. Aeration is completed when the hydrogen peroxide concentration in the system has fallen below 1 ppm, or national limit that needs to be verified with local authorities.

Health and Safety

Hydrogen peroxide is a naturally occurring chemical that is frequently used for medical sterilization purposes. It is stored as a clear liquid, and its vaporized presence is therefore difficult to detect without the use of chemical indicators/sensors. In high concentrations the gas can be extremely toxic, so safe handling is required during fumigation.

When heated, the hydrogen peroxide becomes a vapor and expands to fill voids in the target fumigation area. The expansion properties of vapors are lower than those of gases, therefore, the BSC fans should be operating during the fumigation process. Because of this, a sealed BSC will maintain a negative pressure and contain vaporized hydrogen peroxide quite well. Therefore, it is possible to have an effective and safe containment of the chemical even if there are small gaps in the sealing of the BSC framework. This is a significant advantage of this procedure because access to the complete laboratory room may not necessarily need to be sealed off during the BSC fumigation procedure.

Effectiveness

Hydrogen peroxide used as a vaporized fumigant has been well documented to be effective in achieving a 6-log sporicidal reduction as confirmed using biological indicators.

Material Compatibility

Thermo Fisher Scientific has performed material compatibility testing with hydrogen peroxide fumigation, exposing all BSC components between 500-1200 ppm. Through these studies, material changes on metals, plastics and HEPA filters were not detected. However, when the BSC manufacturer's hydrogen peroxide fumigation process is not followed closely, it is possible for excessive hydrogen peroxide condensation to form inside the BSC, which can damage metal surfaces and coatings.

Ease of Use

The key advantages of this technology are in its ease of use. Cycle parameters can be shortened to as low as 3 hours. BSC set-up time can also be reduced as it is not absolutely critical to achieve an airtight seal of the framework since the fumigation process takes place under negative pressure. Finally, clean up is also shortened since the by-products of the reaction are water and oxygen.

	Formaldehyde	Chlorine Dioxide	Hydrogen Peroxide
Material compatibility	+	-	+/-
Carcinogenic	Yes	No	No
Permissable exposure level (PEL)*	0.75 ppm	0.1 ppm	1 ppm**
Humidity requirements	>60%	60-80%	70-90%
Sealing of cabinet	Airtight, non-authorized personnel should leave room	Airtight, non-authorized personnel should leave room	Small gaps are acceptable when method operates BSC at negative pressure
Generation of gas/vapor	Heated pan or vaporizer to depolymerise the powder form or vaporize a formalin solution	Chemical reaction in a beaker using water and sodium chlorite or by passing chlorine gas through salt sodium chlorite	Generator needed to flash vaporize a hydrogen peroxide solution
Kill time	6-12 hours	60-85 min***	30-110 min***
Deactivation step****	Neutralization with ammonia is required	Gas is catalyzed by charcoal	Hydrogen peroxide vapor will break down naturally over time to oxygen and water but can be accelerated via catalyzer
Clean-up step	It may be necessary to wipe up formaldehyde residue	No	No
Duration of BSC down time	18-24 hours	3 hours	2-3 hours

Table 1: Comparison of fumigation methodologies

* Adapted from Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (IFA) that contains a database of occupational limit values for hazardous substances gathered from various EU member states, Australia, Canada, Japan, New Zealand, Singapore, South Korea, Switzerland, and the United States. For more information visit: http://www.dguv.de/ifa/en/gestis/limit_values/index.jsp

** or according to local authorities

*** dependent upon generator type and method used

**** assumes BSC is not ducted, i.e., gas/vapor cannot be expelled to the outdoors

Preparing a Thermo Scientific Biological Safety Cabinet for Fumigation

Whether your BSC is recirculating or ducted, it is of paramount importance that both the front aperture and the exhaust HEPA filter are sealed prior to any fumigation process commencing.

Thermo Scientific™ Herasafe™ KS, Safe 2020, Herasafe™ KSP and Maxisafe 2020 biological safety cabinets are designed with the SampleGuard aerosol-tight sealing feature. The front window, once closed, seals the front aperture, and the cabinet then enters into a reduced flow mode, keeping a negative pressure within the framework while fumigation takes place. There is no need for any tape or bags to be used around the front window or aperture. For sealing the exhaust HEPA filter, we offer an easy-to-fit gasket sealed top box, which simply sits above the HEPA filter. Preparation for fumigation on a Thermo Scientific BSC is quick and simple.

With Thermo Scientific 1300 Series A2 and MSC-Advantage biological safety cabinets, an optional window sealing kit is available to ensure the cabinet is sealed and ready for fumigation.

The window sealing kit (see figure 1) includes a gasket which is fitted along the top edge of the sash window. The sash window is then lowered and the cabinet will automatically revert to the reduced speed mode.

Also included in the window sealing kit are plastic wedges which are inserted on the left and right sides of the glass to form a compression seal against the window gasket. The lower edge of the window can then be sealed with adhesive tape. Additionally, the 1300 and MSC Advantage biologial safety cabinets will be under negative pressure throughout the fumigation process, preventing any leakage into the laboratory.

Thermo Fisher Scientific also offers a second method for sealing the cabinet while the front sash is open (see figure 2), which is specifically for H_2O_2 fumigation (see table 3). Once the front sash is sealed, it is necessary to seal off the exhaust, which can be facilitated by using an adjustable top box (50135767).

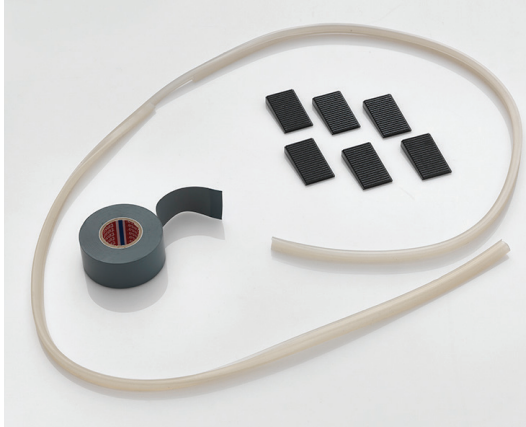


Figure 1: Fumigation window sealing kit (50114036)

Cat. No.	Complete kits
50132757	H_2O_2 adjustable sealing kit (ASK) includes adjustable top box and front window cover. Fits all sizes of Thermo BSC. (includes outlet and inlet connections for STERIS™)
	Inlets only
50135768	H_2O_2 front window sealing plate. This seals the front aperture, and has a STERIS connector
50137105	STERIS H_2O_2 Camlock inlet connector. Fits into one access port left or right side of the BSC
50137107	Bioquell™ H_2O_2 Camlock inlet connector (includes sample connector). Uses two access ports on side of BSC
	Outlets only
50137106	STERIS or Bioquell Camlock outlet connector (for connection to top box or duct connectors)
50135767	H_2O_2 adjustable top box for Thermo Scientific BSC with Outlet connector (STERIS or Bioquell)
	Others
50114036	Fumigation window sealing kit for MSC Advantage/1300 series only

Table 2: Fumigation accessories

H_2O_2 Fumigation Accessories

When fumigating a biological safety cabinet using H_2O_2, there are many accessories available. When choosing accessories there are basic questions to consider. See the chart below for guidance.

- Is the biological safety cabinet recirculating or ducted (with a KDD connector)?
- Is the inlet of the H_2O_2 generator connected via a removable front sealing gasket (STERIS only) or through a fixed side connector (STERIS or Bioquell)?

Figure 2: Adjustable sealing kit (50135768)

	Recirculating		Ducted	
	Side Connection	Front Connection	Side Connection	Front Connection
STERIS	50137105 (one per cabinet) 50135767 (one required)	50132757 (one required)	50137105 (one per cabinet) 50137106 (one per cabinet)	50135768 (one required) 50137106 (one per cabinet)
Bioquell	50137107 (one per cabinet) 50135767 (one required)	Not available	50137107 (one per cabinet) 50137106 (one per cabinet)	Not available

Table 3: Choosing your H_2O_2 fumigation accessories

Technical Note

Thermo Scientific Biological Safety Cabinets

protection

that never takes a day off

With Thermo Scientific biological safety cabinets, the certified performance and protection you get on Day 1 stays with you everyday. Not true with ordinary cabinets. The difference is our design: SmartFlow™ technology features dual-DC motors to automatically balance the cabinet inflow and downflow air velocities in real time – even as the filters load.

That means exceptional user and sample protection you never have to think about. Plus, our Digital Airflow Verification (DAVe) alarm signals any out-of-spec conditions for added assurance. Combined with our proven reliability, ergonomics and energy efficiency, the ideal choice is also the one you can trust completely. And not just on Day 1.

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